

Executive Summary

The United States Fish and Wildlife Service's (USFWS) National Investigational New Animal Drug Office in Bozeman, MT, conducted a study (AQUIS-01-EFF.2-13) under Study Protocol Number AQUIS-01-EFF to generate efficacy data needed to help obtain U.S. Food and Drug Administration (FDA) approval for the use of AQUI-S® as a fish anesthetic. Study AQUIS-01-EFF.2-13 (conducted September 14 - 16, 2004) consisted of four experiments, in each of which three AQUI-S® "treatments" (20, 40, and 60 mg/L) and one Tricaine-S® (MS-222) "control" (80 mg/L) were used to test largemouth bass *Micropterus salmoides* (LMB; test fish) to and from the "handleable" stage of anesthesia. In Experiments 1 (juvenile test fish) and 2 (adult), fish were tested at a median water temperature of about 18°C. In Experiments 3 (juvenile) and 4 (adult), fish were tested at a median water temperature of about 12°C. In all four experiments, mean AQUI-S® doses delivered were analytically verified and were all within $\pm 25\%$ of target doses.

In each experiment, the AQUI-S® treatments and MS-222 control were each administered to 15 test fish. Consequently, 60 test fish were used in each experiment; and a total of 240 test fish were used in the study. Fish were tested individually to handleable (60-min maximum-time limit allowed), and only fish that became handleable within 60 min were tested to recovery (30-min maximum-time limit allowed). All fish,

including those that did not become handleable within 60 min, were monitored for survival.

All AQUIS[®]-treated fish (overall median length of juveniles = 18.0 cm; overall median length of adults = 36.5 cm) were tested in anesthetic solutions prepared immediately before use. Based on data collected on all AQUIS[®]-treated fish tested to handleable, test fish behavior was characterized as normal for 100% of the juvenile and adult fish tested at either 12 or 18°C. There appeared to be no lasting effects of the AQUIS[®] treatments, and no AQUIS[®]-treated fish died during the study. Based on data collected on all AQUIS[®]-treated fish when tested to recovery from handleable, test fish behavior was characterized as normal for 100% of the fish tested at both 12 and 18°C.

In all experiments, 100% of the 15 test fish in each AQUIS[®] treatment group became handleable within 60 min. Based on data collected at 20, 40, and 60 mg/L AQUIS[®], median times for juveniles to become handleable ranged from 1.35 to 6.75 min, and median times for juveniles to recover ranged from 2.50 to 5.48 min. Median times for adults to become handleable ranged from 2.55 to 6.55 min, and median times for adults to recover ranged from 2.62 to 8.47 min. In all four experiments, median time to handleable decreased as AQUIS[®] concentration increased, and Kaplan-Meier (K-M) survival analysis confirmed that in all experiments the time to handleable decreased significantly as AQUIS[®] concentration increased from 20 to 40 to 60 mg/L. No such trend was observed with respect to median time for fish to recover from handleable and

concentration of AQUIS-[®] used to anesthetize fish. All test fish were returned to the reference population after exposure and recovery.

Comparisons within AQUIS-[®] treatments were made between Experiments 1 and 2 and Experiments 3 and 4 to investigate possible life-stage effects on times to and from handleable when median water temperature was held constant at either 18 or 12°C. Comparisons of median times to and from handleable indicate that juveniles become handleable faster and recover from handleable faster than adults at both temperatures, but K-M analysis of time-to-handleable data only confirmed significant differences in times for fish exposed to 40 and 60 mg/L.

Within-treatment comparisons were made between Experiments 1 and 3 and Experiments 2 and 4 to investigate possible water temperature effects on times to and from handleable when life-stage was held constant at either juvenile or adult. Comparisons of median times to and from handleable indicate, and K-M analysis confirmed, that juveniles become handleable and recover from handleable faster at the warmer water temperature (i.e., about 18°C) than at the cooler water temperature (i.e., about 12°C). No such trend was observed between median times to and from handleable or recovery from handleable for adult fish.

Overall, the AQUIS-[®] efficacy data generated during study AQUIS-01-EFF.2-13 support the following conclusions:

- 1.** AQUIS-[®] concentrations of 20, 40, and 60 mg/L are efficacious and safe for inducing juvenile and adult largemouth bass to handleable at water temperatures of about 12 and 18°C. Such a use of AQUIS-[®] will be acceptable to most fisheries professionals because (a) “handleable” induction and recovery times fall within the guidelines of an “ideal” fish anesthetic, and (b) no adverse effects were observed;
- 2.** Regardless of water temperature, the time required for juvenile and adult largemouth bass to become handleable will decrease as AQUIS-[®] concentration is increased from 20 to 40 to 60 mg/L;
- 3.** At a given water temperature and AQUIS-[®] concentration, juvenile largemouth bass will likely become handleable faster and recover from handleable faster than adult largemouth bass; and
- 4.** At different water temperatures and a given AQUIS-[®] concentration, juvenile largemouth bass will likely become handleable and recover from handleable faster in warmer water than in cooler water.

Table 4. Median time to handleable (HT) and recovery from handleable (HRT) for (a) juvenile largemouth bass tested at about 18°C, (b) adult largemouth bass tested at about 18°C, (c) juvenile largemouth bass tested at about 12°C, and (d) adult largemouth bass tested at about 12°C.

| Anesthetic concentration (mg/L) | Juvenile at about 18°C | | Adult at about 18°C | | Juvenile at about 12°C | | Adult at about 12°C | |
|---------------------------------|------------------------|-----------|---------------------|-----------|------------------------|-----------|---------------------|-----------|
| | HT (min) | HRT (min) | HT (min) | HRT (min) | HT (min) | HRT (min) | HT (min) | HRT (min) |
| AQUI-S® | | | | | | | | |
| 20 | 5.48 | 2.50 | 6.08 | 2.62 | 6.75 | 3.85 | 6.55 | 4.45 |
| 40 | 1.57 | 2.97 | 4.87 | 7.57 | 2.45 | 4.83 | 4.37 | 6.75 |
| 60 | 1.35 | 3.28 | 2.55 | 6.73 | 2.43 | 5.48 | 2.88 | 8.47 |
| MS-222 | | | | | | | | |
| 80 | 1.97 | 1.45 | 3.05 | 1.95 | 2.33 | 1.67 | 2.83 | 2.83 |